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(12) UK Patent Application (19) GB (11) 2 206 582 (13) A

(43) Application published 11 Jan 1989

(21) Application No 8813095

(22) Date of filing 3 Jun 1988

(30) Priority data
(31) 62/139021 (32) 4 Jun 1987 (33) JP
63/096870 21 Apr 1988

(51) INT CL⁴
C07H 3/06 C08B 37/00

(52) Domestic classification (Edition J):
C2S 107 120 340 360 EX
A5B 180 230 23X 23Y 827 830 KC
C3U 219 AD
U1S 1075 1317 1318 A5B C2S C3U

(56) Documents cited
None

(58) Field of search
C2S
C3U
Selected US specifications from IPC sub-classes
C08B C13K C07H

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# (54) Palatinose condensation product and a process for the preparation thereof and a method for utilizing the product

(57) A palatinose condensation product having 2 to 8 palatinose units is disclosed together with processes for its preparation and uses as a foodstuff, and a medicament for encouraging the growth of the beneficial micro-organism *Bifidobacterium* in the intestines. The medicament may also contain palatinose.



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FIG. 1

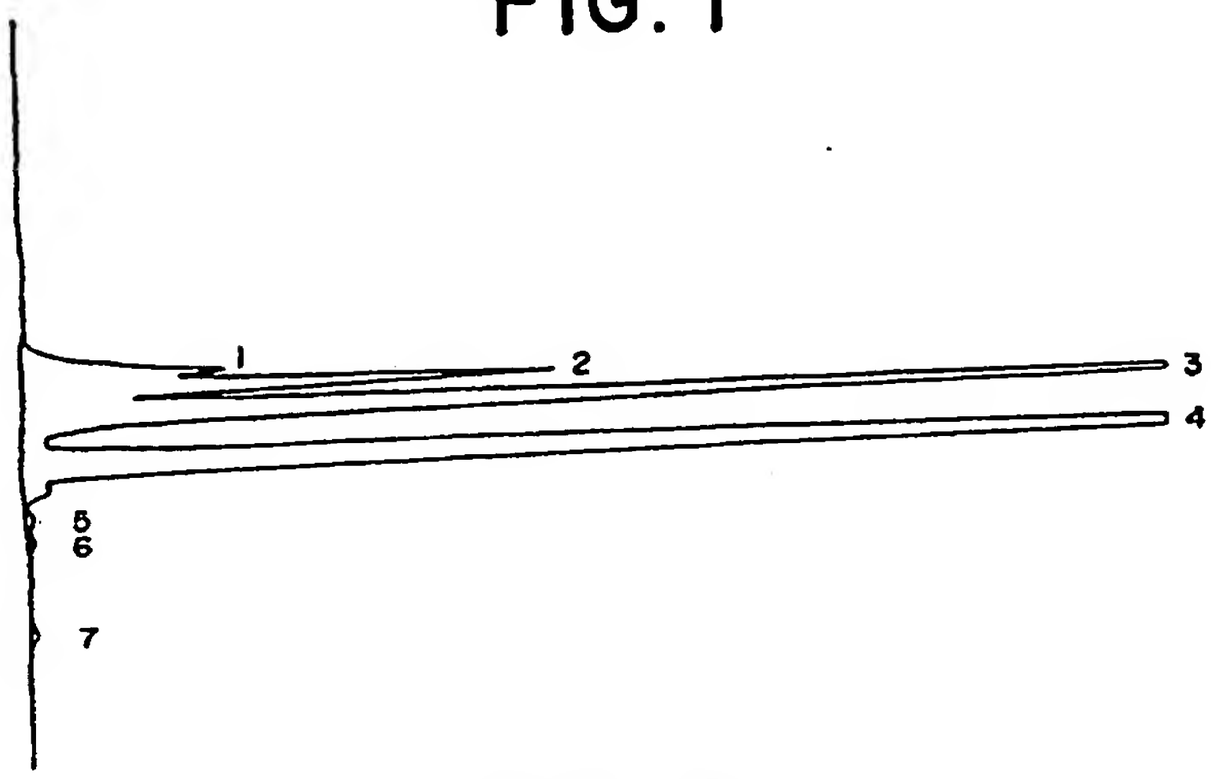



FIG. 2

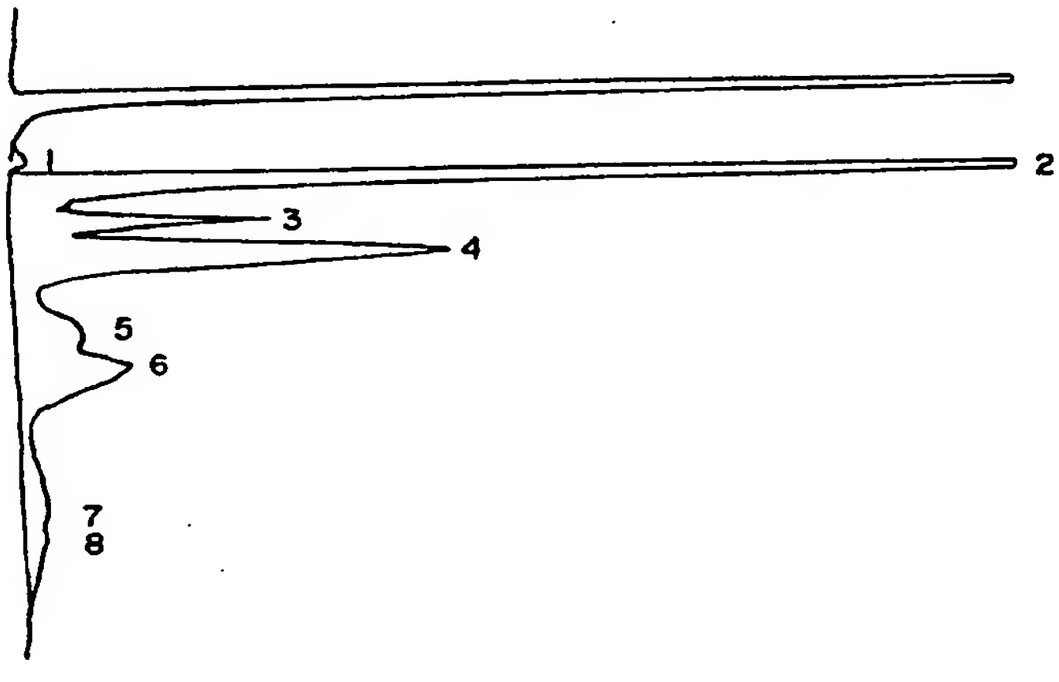



FIG. 3

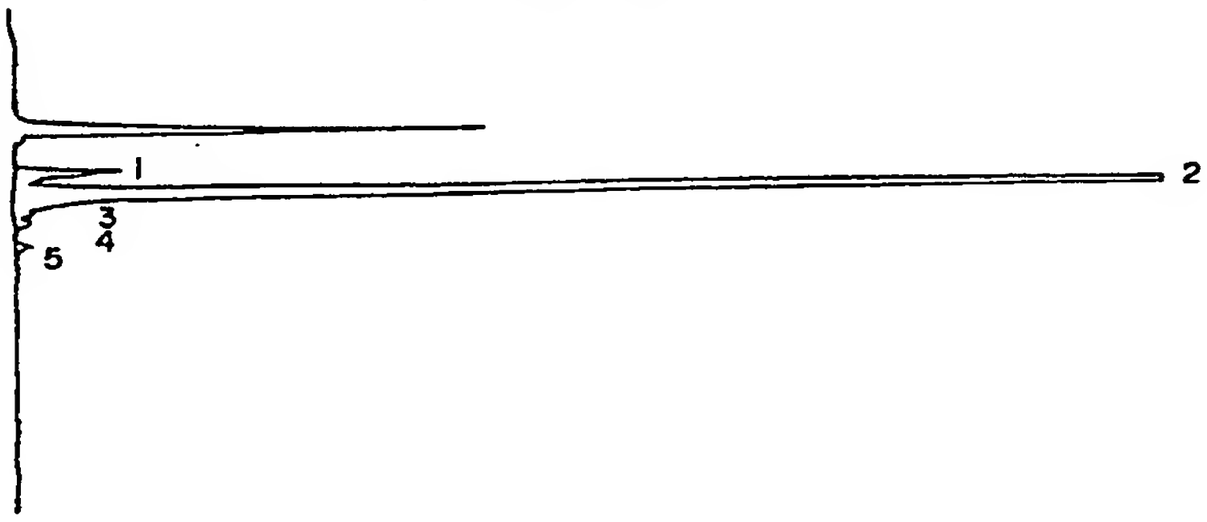
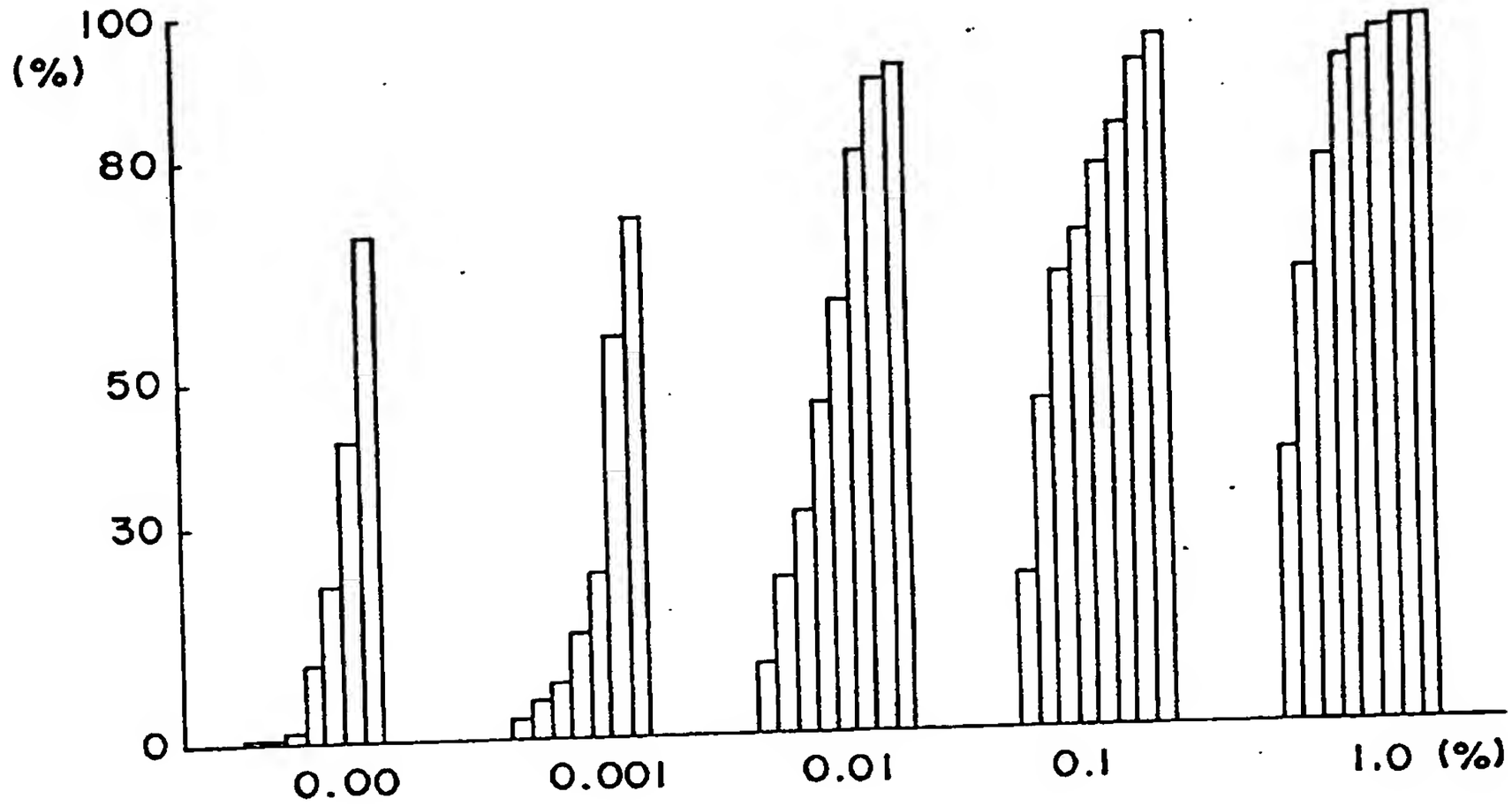


FIG.4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30
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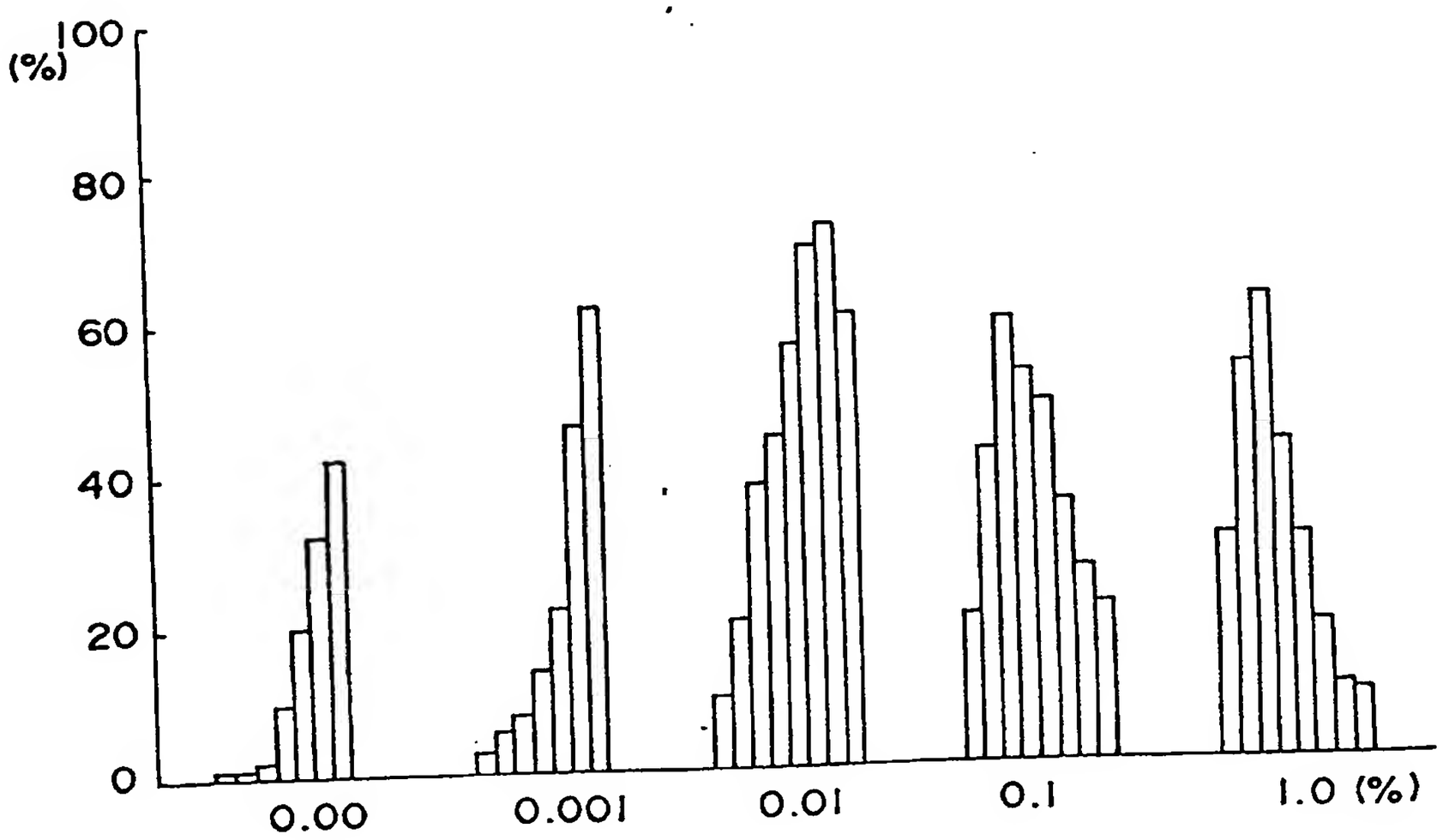
0.00
0.001
0.01
0.1
1.0 (%)

FIG.5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40
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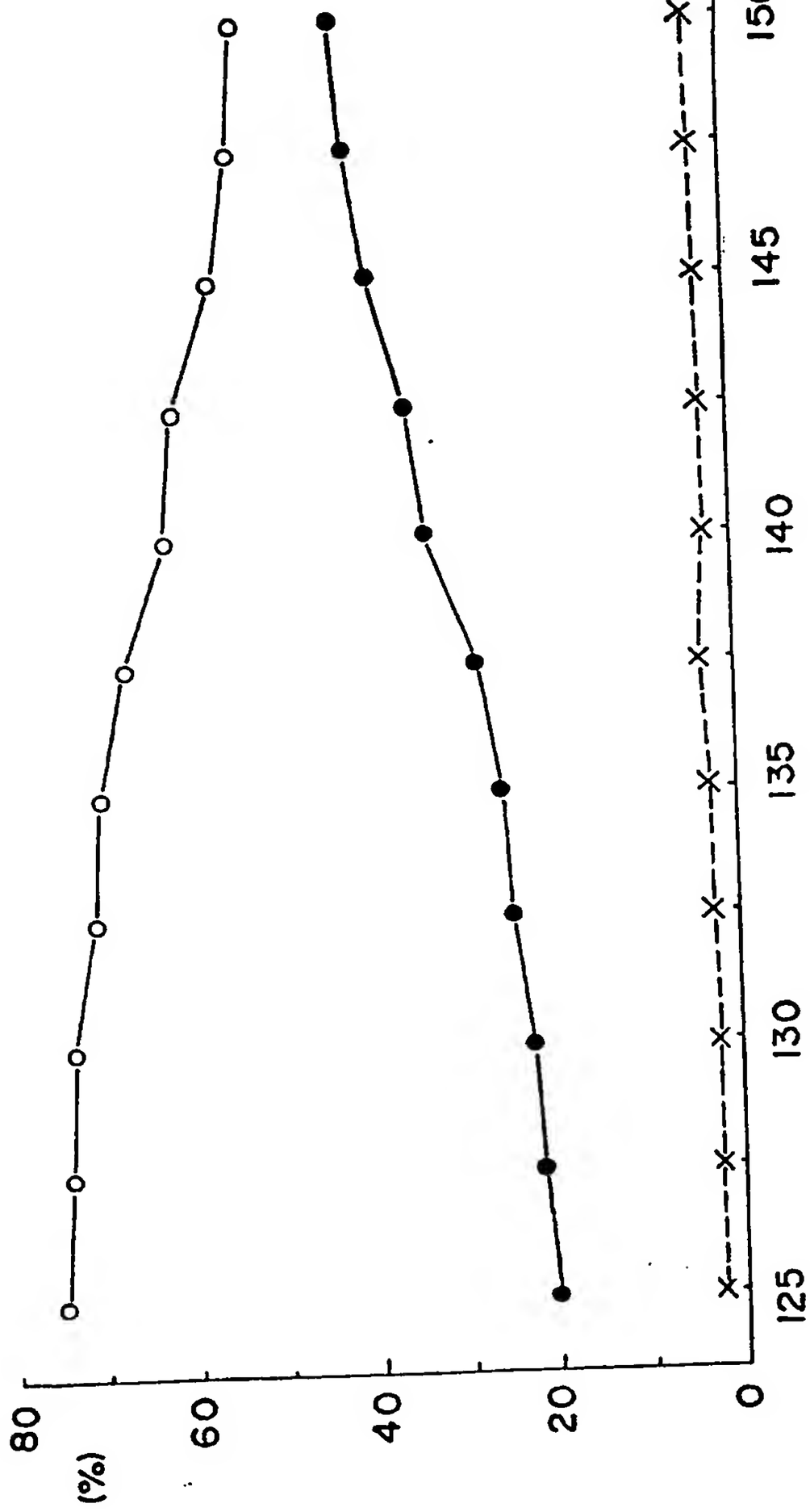


FIG. 6

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# "PALATINOSE CONDENSATION PRODUCT AND A PROCESS FOR THE PREPARATION THEREOF AND A METHOD FOR UTILIZING THE PRODUCT"

This invention relates to a palatinose condensation product which is obtained by the condensation of disaccharide palatinose, a process for the preparation of it and a method for utilizing it.

Palatinose, which is also called isomaltulose, is a disaccharide in which glucose and fructose are α-1,6-bonded, i.e. 6-o-α-D-glucopyranosyl-fructofuranose. Palatinose per se has been known for many years and is a glycide contained in honey in nature, and is obtained in industry by having glucosyltransferase act on sucrose (DE 3133128, UK 2082591 and US 4386158). Palatinose has good quality of sweetness and good processability for foods similar to those of sucrose, which makes it possible to use palatinose in foods generally. Particularly because palatinose is non-cariogemic and anti-cariogenic, i.e. preventing cariogenicity of sucrose, the demand for palatinose has rapidly been increasing in recent years (DE 3142093, UK 2086203 and US 4556429).

Further a method is known for preparing a fructose condensation product by concentrating and then heating a solution of monosaccharide fructose at an acidic pH (Japanese Patent Application Laid-Open 86/271295). This fructose condensation product is a sweetening. It is also known to utilize the fructose condensation product for proliferating Bifidobacterium (Japanese Patent Application Laid-Open 87/294081).

The present inventors have now prepared a palatin-

ose condensation product from disaccharide palatinose through condensation, and have found that the condensation product has properties very useful in the food industry.

It has now been found that when an aqueous palatinose solution having a pH of 3.2 to 5.8, preferably a pH of 3.5 to 4.4 is heat-concentrated up to a liquid temperature of about 105 to 170°C, palatinose condenses into a condensation product composed of 2 to 6, mainly 2 to 4, palatinose units.

The obtained palatinose condensation product is a novel compound and is found to be capable of being used instead of conventional thick malt syrup in foods to raise viscosity, to prevent freezing, to improve moisture retention in food, to attain a body effect and to suppress the proliferation of putrefying bacteria. The reaction mixture of palatinose condensation which contains a substantial amount of the palatinose condensation product may also be used as a substitute for conventional thick malt syrup.

Further, the palatinose condensation product is very useful to proliferate intestinal Bifidobacterium.

The process for the preparation of the palatinose condensation product and the resulting condensation product will now be described.

The condensation reaction is greatly influenced by the pH, temperature and concentration of the aqueous palatinose solution. The reaction is facilitated by higher temperature, lower pH and higher concentration. When these factors are in conditions such as to strongly facilitate the reaction, the formation of the palatinose condensation product increases, and also increased are a degradation product and monosaccharide

formed by decomposition of palatinose, and a trisaccharide-corresponding product which seems to be a condensation product of these with palatinose.

The palatinose condensation product is colourless and has considerably low sweetness, while the degradation product formed by decomposition of palatinose has bitterness. Accordingly, when a reaction mixture of the palatinose condensation reaction is used as such, i.e. without isolating the condensation product, in foods, it is preferred to adjust condensation reaction conditions so as to suppress the formation of the degradation product below a certain amount, preferably 0.45 wt.% or less, or to remove the degradation product from the condensation reaction mixture with an exchange resin. The bitterness may be eliminated by adding an alkaline substance to the reaction mixture to make it neutral or alkaline. However, discolouring becomes noticeable.

It has now been found that it is preferred to conduct the heat concentration in reduced pressure conditions, particularly about 110 mmHg (absolute, hereinafter the same) or below, for a relatively short time in order to suppress the formation of the degradation product. The heating time depends upon the apparatus used, but is generally 1 to 60 minutes. It is preferred to continue the concentration until the water content becomes 1 to 5% by weight. The liquid temperature at the end of the reduced-pressure concentration should be about 105 to 170°C, preferably 105 to 160°C, particularly 130 to 160°C. For instance, a concentrator of the spirally descending thin film type or an evaporator of the continuous concentration type is suitable for use in the reduced-pressure

concentration procedure, where the contact time of the feed with the heating surface is short.

The heat concentration can however be carried out at normal pressure. Generally, it is necessary to raise the liquid temperature up to high temperatures of 140 to 165°C in order to attain a water content suitable for the condensation reaction. Further, the heating time is also relatively long. Therefore, the palatinose degradation product is formed in a larger amount. A method of raising the temperature by the use of a plate type heat exchanger and flashing the liquid is advantageous in that the time for which the liquid is maintained at high temperatures is short, but the degree of concentration is insufficient and the water content in the liquid is relatively high, so that the condensation reaction tends not to proceed sufficiently.

Further, it may be thought to use an extruder as a pressurizing melting method, but this is however disadvantageous in the formation of the degradation product. Any of these methods may cause the condensation reaction of palatinose.

A palatinose solution in water shows a pH value around 5.8. The condensation reaction of palatinose is facilitated by acid catalysts. Therefore, an acid is preferably added to the starting palatinose solution in water to adjust the pH to about 3.2 to 5.8, preferably 3.5 to 4.4. The acid to be used is not particularly restricted, but those used generally in foods may be preferably used, for instance less volatile organic acids such as citric acid, malic acid, succinic acid and tartaric acid. Citric acid is particularly preferred because its catalytic activity is relatively

high and the amount of the resulting degradation product is less. More volatile organic acids such as acetic acid will vaporize into air during the concentration process and therefore have to be replenished, which is unfavourable in operation. Inorganic acids such as hydrochloric acid, phosphoric acid and sulphuric acid show a little worse reproducibility of the reaction compared to the organic acids, which leads to unstable quality of the product. Therefore, the inorganic acids are not always preferred. Two or more acids may be used together.

The concentration of palatinose in the starting aqueous palatinose solution is not particularly restricted, and may be in a range where palatinose is substantially completely dissolved during the heating. Too low concentrations require a prolonged time period of the concentrating operation and therefore are disadvantageous. In general, concentrations of about 70 to 80% by weight are preferred.

After the condensation reaction is conducted as explained above, a reaction mixture with a solid content of 95 to 99% by weight is obtained. The reaction mixture contains tetra- to dodeca-saccharide, mainly tetra- to octa-saccharide, resulting from condensation of 2 to 6, mainly 2 to 4, palatinose units generally in an amount of 10 to 70% by weight. Monosaccharide resulting from the decomposition of disaccharide palatinose, trisaccharide-corresponding products which are thought to be caused by the condensation of palatinose with the above monosaccharide or with its derivatives in which the ring is opened (hereinafter referred to simply as trisaccharide) and the degradation product are formed generally in an

amount of 20% by weight or less in all. Further, unreacted palatinose remains therein, of course.

The properties and utilization of the palatinose condensation product of the invention will now be described.

The palatinose condensation product of the invention has much lower sweetness than that of sucrose and, even, that of palatinose. This condensation product reverts to palatinose upon hydrolysis and, therefore, is non-cariogenic and anti-cariogenic like palatinose. Accordingly, the palatinose condensation product may be used to modify texture, etc. of foods without changing the sweetness of the foods to which it is added and is particularly useful in foods characterized by non-cariogenicity or anti-cariogenicity. Above all, it exhibits a special effect when it is used in foods in which palatinose is contained as a sweetening. That is, palatinose is less soluble compared to sucrose, which limits the use of palatinose. For instance, it is impossible to produce jam, sweet jelly of beans, hard jelly or caramel due to the precipitation of palatinose crystals called "revertion" or "gritty". In order to prevent "revertion" or "gritty", it may be thought to add sorbite, hydrolised starch, reduced maltose syrup, sucrose, coupling sugar or the like. However, in such a method, the amount of palatinose contained in confectionery is decreased inevitably and, then, such confectionery cannot be described as having less cariogenicity or as being non-cariogenic. It has now been found that when the palatinose condensation product of the invention is used together with palatinose in foods, precipitation of palatinose crystals may be inhibited or prevented and

such foods can be described as low cariogenic foods.

When the palatinose condensation product is used together with palatinose according to the invention, it is not always necessary to separate the palatinose condensation product from the palatinose condensation reaction mixture. It is possible to use a reaction mixture as a whole which contains unreacted palatinose, trisaccharide, etc. besides the palatinose condensation product (hereinafter referred to as a product mixture), wherein the remaining palatinose may be taken into consideration in a recipe. In such a case, it is generally recommendable to use a product mixture which contains a less amount, e.g. 0.45 wt.% or less, of the aforesaid bitter degradation product, though the numeric value, "0.45 wt.%", is merely illustrative because bitterness depends also upon the amount of the product mixture added to a food.

Further, the palatinose condensation product or the product mixture according to the invention may be used as a substitute for conventional malt syrup. That is, these materials have the effect of raising the viscosity, preventing freezing, improving moisture retention in food, attaining a body effect and suppressing the proliferation of putrefactive bacteria.

The palatinose condensation product of the invention is also very usetful as a substance enhancing the proliferation of Bifidobacterium, which will be explained below.

A lot of bacteria inhibit the human intestines soon after birth. The number of bacteria living in intestines of a single person is $10^{x14}$ or more and the number of the species of the bacteria is almost 100. These repeat the proliferation making use of the

contents of the intestines as nutrients to establish intestinal flora while keeping a certain balance. Anaerobic bacteria such as Bacteroidaceae, Eubacterium and anaerobic Streptococcus, and Bifidobacterium important as human lactic acid bacterium are known as dominant bacteria in adult intestines. Those bacteria inhabit the intestine whilst keeping a balance among themselves. Recently, a method of cultivating the intestinal bacteria was developed and, accordingly, researches on the intestinal bacteria have actively been made to find that some of those intestinal bacteria are useful to a host and some others enter into the tissue of a host to cause damage to it or produce noxious substances. Thus, the intestinal bacteria have been drawing attention. The number of enzymes born by the intestinal bacteria is said to be larger than that of enzymes of a liver. It is also said that the intestinal bacteria have great influence on human bodies and the intestinal flora even may effect whether a person is healthy. Bifidobacterium is known as a physiologically favourable bacteriaum effective to maintain health, which always inhabit the intestines of infants and adults and show antagonistic action against the proliferation of putrefactive bacteria, prevention of intestinal absorption of noxious substances, enhancement of immunological functions and improvement of metabolism of vitamins. Further, this bacterium is said to have a close relationship with aging. In order to make efficient use of such usefulness of Bifidobacterium, it is desired to always maintain Bifidobacterium at a high level in intestines, and medicaments and foods containing Bifidobacterium are now available on the market.

Temporary increase of Bifidobacterium can be attained by orally administering Bifidobacterium continuously. However, if the administration is stopped, it is eliminated from the body for a short time and the desired effect is not attained. Then, it has been thought important to create environments where Bifidobacterium stays and proliferates, and some attempts have been made to maintain the number of the intestinal Bifidobacterium at a high level by orally administering a substance which enhances the proliferation of Bifidobacterium. Recently, a factor most necessary for Bifidobacterium to proliferate in intestines was thought to be saccharide as an energy source, and it was in fact found that Bifidobacterium could exploit raffinose, stachyose, inulin etc. among oligosaccharides and polysaccharides. Based on this fact, it was proposed that lactulose, raffinose, stachyose, inulin, fructooligomer, isomaltooligomer, galacturose and galactooligosaccharide are effective to proliferate Bifidobacterium in host intestines. However, some of these saccharides show poor assimilation by Bifidobacterium and some others exhibit a poor effect on Bifidobacterium in practical administration to a human being, or lack selectivity to intestinal bacteria being exploited by unfavourable intestinal bacterium as well as Bifidobacterium, or generate gas extraordinarily. Therefore, it has been needed to develop a useful saccharide source which is specifically utilized by Bifidobacterium and has no side effects.

It has now been found that the palatinose condensation product is very effective to selectively enhance the proliferation of Bifidobacterium, which is another aspect of the invention.

comprising the palatinose condensation product in an effective amount.

The composition according to the invention includes any of the product mixture per se which is prepared as described above and contains 10 to 70 wt.% of the palatinose condensation product, or foods, drinks or medicaments or their raw materials to which the palatinose condensation product or the product mixture is admixed. Preferably, the palatinose condensation product is contained in the composition useful in the proliferation of Bifidobacterium in an amount of about 0.5 to 80% by weight, particularly about 1 to 70% by weight. If it is less than about 0.5% by weight, the effect on the proliferation of Bifidobacterium is poor. On the other hand, an amount more than about 80% by weight does not cause any particular problem. However, when it is utilized in foods, there is some limitations from the viewpoint of taste and production process and the amount is about 70% by weight or less in general.

The invention may be put into practice in various ways and a number of specific embodiments will be described by way of example to illustrate the invention with reference to the accompanying drawings, in which:

Figures 1 and 2 are high performance liquid chromatographs of a palatinose condensation reaction mixture with different columns;

Figure 3 is a high performance liquid chromatograph of a hydrolyed reaction mixture;

Figures 4 and 5 are graphs of conversions of palatinose and yields of the condensation product, respectively, at different amounts of citric acid and different temperatures; and

Figure 6 is a graph showing compositions of the reaction mixtures at different temperatures.

In the Examples, "%" and "part" means weight percent and weight part, respectively, unless otherwise stated.

## EXAMPLE 1

Preparation of the palatinose condensation product.

A hundred (100) parts of crystalline palatinose and 0.01 part of anhydrous citric acid were added to 33 parts of boiling water and heated to 105°C with stirring to dissolve the materials. The mixture was then heated to 150°C within 1 minute under a pressure of 60 mmHg in the heating tubes of a vacuum continuous evaporator and, then, subjected to the concentration and reaction for about 3 minutes in the vacuum evaporator. Subsequently, it was solidified into pieces of 6g apiece with a stamping machine and crushed with a crusher. The resulting product containing the palatinose condensation product had the following composition.

The resulting product had a solid content of 99.0%, pH of 4.2 and a colour value of 31 in ICUMSA units, and exhibited no bitterness and a little sweetness. The resulting product was subjected to high performance liquid chromatography as stated above and was found to contain 44.3% of the palatinose condensation product, provided that the water contained was not taken into the calculation of composition.

Analysis of the composition of the obtained reaction mixture will be described. The reaction mixture was subjected to high performance liquid chromatography under the following conditions.

was subjected to high performance liquid chromatography under the following conditions.

Column: Shodex Ionpak KS-802
Moving phase: water
Flow of the moving phase: 1 ml/min.
Detector: Differential refractometer
Sensitivity of the differential refractometer: 16 x
Chart speed: 0.5 cm/min.
Temperature: 80°C
Concentration of the sample: 5 w/v %
Injected amount: 25 microlitre.

Figure 1 is the chromatograph which was obtained. The following Table 1 gives the species corresponding to the retention time of each peak and its amount.

TABLE 1

| Peak No. | Retention time | Species | Amount % |
|---|---|---|---|
| 1 | 5.824 | palatinose condensation product octasaccharide | 5.0 |
| 2 | 6.053 | ditto hexasaccharide | 11.5 |
| 3 | 6.449 | ditto tri- and tetra-saccharide | 30.6 |
| 4 | 7.249 | palatinose | 52.2 |
| 5 | 8.135 | glucose | 0.2 |
| 6 | 8.480 | fructose | 0.2 |
| 7 | 9.410 | degradation product | 0.3 |

In Figure 1, the fractionation between trisaccharide and tetrasaccharide is insufficient (peak No. 3). Because of this the same sample was then subjected to high performance liquid chromatograph under the following conditions which were suited to this fractionation

but insufficient for the fractionation of monosaccharides.

Column: ERC-NH-1171 (Elmor Optics Co.)
Moving phase: acetonitrile/water = 63/37
Flow of the moving phase: 1 ml/min.
Detector: Differential refractometer
Sensitivity of the differential refractometer: 16 x
Chart speed: 0.5 cm/min.
Temperature: 25°C
Concentration of the sample: 5 w/v %
Injected amount: 25 microlitre.

Figure 2 is the chromatograph which was obtained. The following Table 2 gives the species corresponding to the retention time of each peak and its amount.

TABLE 2

| Peak No. | Retention time | Species | Amount % |
|---|---|---|---|
| 1 | 6.270 | monosaccharide | 0.4 |
| 2 | 6.963 | palatinose | 54.0 |
| 3 | 8.596 | palatinose condensation product trisaccharide | 3.0 |
| 4 | 9.870 | ditto tetrasaccharide | 29.8 |
| 5 | 12.950 | ditto hexasaccharide | 4.0 |
| 6 | 14.050 | ditto hexasaccharide | 7.3 |
| 7 | 19.470 | ditto octasaccharide | 0.9 |
| 8 | 20.830 | ditto octasaccharide | 0.6 |

From the above, it was found that the ratio of the trisaccharide to tetrasaccharide was about 1 to 10. Accordingly, by proportionally dividing the peak No. 3 in Table 1, the following composition given in Table 3 can be deduced.

TABLE 3

| Species | | | Ratio (%) |
|---|---|---|---|
| Palatinose condensation product | | octasaccharide | 5.0 |
| | ditto | hexasaccharide | 11.5 |
| | ditto | tetrasaccharide | 27.8 |
| | ditto | trisaccharide | 2.8 |
| | | | 52.2 |
| Palatinose | | | 0.2 |
| Glucose | | | 0.2 |
| Fructose | | | |
| Degradation product | | | 0.3 |

Thus, the sample contained 44.3% of tetra- to octasaccharide of the invention.

Next, the heat concentration product was subjected to hydrolysis and analysed. That is, a solution in water was prepared with a solid content of 5% of the heat concentration reaction mixture obtained in Example 1 above, to 10 ml of which 1 ml of 1 N hydrochloric acid was added, heated at 80°C for 15 minutes and neutralised. The resultant product was subjected to high performance liquid chromatography on the same conditions as those of the aforesaid Shodex Ionpak KS-802 used to produce Table 1.

The chromatograph as shown in Figure 3 was obtained.

The following Table 4 gives the species corresponding to each peak and its amount.

TABLE 4

| Peak No. | Retention time | Species | Amount % |
|---|---|---|---|
| 1 | 6.454 | palatinose condensation product trisaccharide | 2.9 |
| 2 | 7.254 | palatinose | 96.0 |
| 3 | 8.140 | glucose | 0.4 |
| 4 | 8.485 | fructose | 0.4 |
| 5 | 9.415 | degradation product | 0.3 |

It is clear that the palatinose condensation product of the invention change mostly into palatinose by hydrolysis.

## EXAMPLE 2

Preparation of the palatinose condensation product.

A hundred (100) parts of crystalline palatinose and 0.02 part of anhydrous citric acid were added to 33 parts of boiling water and heated to 105°C with stirring to dissolve the materials. The mixture was then boiled down under a pressure of 60mmHg in a vacuum concentrate until the temperature of the concentrated reached 150°C for 35 minutes. Subsequently, the heating was stopped and the product was solidified into pieces of 6g apiece with a stamping machine and crushed with a crusher.

The resulting product had a solid content of 99.0%, pH of 4.0 and a colour value of 88 in ICUMSA units.

High performance liquid chromatography gave the following composition, wherein the percentages were calculated neglecting the water contained in the

concentrated product.

| | |
|---|---|
| Palatinose condensation product | 70.8% |
| Palatinose | 22.1% |
| Trisaccharide | 6.0% |
| Monosaccharide | 0.6% |
| Degradation product | 0.5% |

This product mixture had slight bitterness and pale yellow discolouring and could be used in any foods.

## EXAMPLE 3

Preparation of the palatinose condensation product.

A hundred (100) parts of crystalline palatinose and 0.05 part of anhydrous citric acid were added to 33 parts of boiling water and heated to 105°C with stirring to dissolve the materials. The mixture was then subjected to reaction under a pressure of 60 mmHg as in Example 1. Subsequently, it was solidified into pieces of 6g apiece with a stamping machine and crushed with a crusher.

The resulting product had a solid content of 99.1%, pH of 3.8 and a colour value of 30 in ICUMSA units, and exhibited almost no bitterness. The composition was as follows, wherein the water was not taken into the calculation.

| | |
|---|---|
| Palatinose condensation product | 66.6% |
| Palatinose | 28.9% |
| Trisaccharide | 3.6% |
| Monosaccharide | 0.5% |
| Degradation product | 0.4% |

EXAMPLE 4

Preparation of the palatinose condensation product.

A hundred (100) parts of crystalline palatinose were added to 33 parts of boiling water and concentrated with stirring for about 10 minutes at normal pressure in a copper pan until the temperature of the contents reached 160°C. It was then solidified into pieces of 3g apiece with a stamping machine.

In the present Example, the heat concentration was carried out at atmospheric pressure, and the concentration was set relatively short to lower the amount of the degradation product.

The resulting product had a solid content of 97.0%, pH of 4.3 and a colour value of 287 in ICUMSA units, i.e. considerable discolouration, and exhibited almost no bitterness and a taste like a candy made of heat-melted crystal sugar. The composition was as follows, the water contained in the resulting product not being taken into account in the calculation.

| | |
|---|---|
| Palatinose condensation product | 18.1% |
| Palatinose | 77.7% |
| Trisaccharide | 2.9% |
| Monosaccharide | 1.0% |
| Degradation product | 0.3% |

EXAMPLE 5

Preparation of the palatinose condensation product.

Thirty (30) kg of crystalline palatinose and 9 kg of water were placed in a 50 l stainless steel vessel and heated to 105°C with stirring to dissolve the materials. The whole amount of this palatinose solu-

tion in water was transferred to a vacuum cooker with a stirrer, to which 3.0 g of anhydrous citric acid were added and the mixture stirred. The pH of the aqueous palatinose solution at this stage was 4.1. Then, the pressure was reduced to 60 mmHg and the content was concentrated with heating. It took 30 minutes for the temperature of the concentrated liquid to reach 135°C. At the moment when the temperature reached 135°C, the heating was stopped. The resulting product had a solid content of 98% and the following composition, the water contained in the resulting product not being taken into account in the calculation.

| | |
|---|---|
| Palatinose condensation product | 53.4% |
| Palatinose | 42.0% |
| Trisaccharide | 3.6% |
| Monosaccharide | 0.6% |
| Degradation product | 0.4% |

EXAMPLE 6

This was a batch process. 15 Kg of a 75 wt.% palatinose solution in water were placed in a 20 l stainless steel kettle concentrator with a stirrer used in the production of hard candy. A final heating temperature was selected in the range of from 100°C to 170°C with an interval of 10°C. The amount of citric acid was 0%, 0.001%, 0.01%, 0.1% or 1.0% by weight. In every case, the pressure was 50 mmHg and the heating time was about 25 to 30 minutes. The results are as shown in Figures 4 and 5. In Figure 4, the ordinate is percentage decrease of palatinose, i.e. conversion, and the abscissa is the amount of citric acid added in percentage calculated on the solid. In Figure 5, the ordinate is the amount of the formed palatinose conden-

sation product, i.e. yield, and the abscissa is the amount of citric acid added. In each group of the bars, the final heating temperature was 170, 160, 150, 140, 130, 120, 110 and 100°C from the right-hand most bar to the left-hand most. In the case were bars on the lower temperature side are not seen, this means that the resultant numeric values were nearly zero. The larger the amount of citric acid added and the higher the reaction temperature, the larger was the conversion of the palatinose. The yield of the condensation product was largest when the amount of citric added was 0.01% and the temperature was 150 to 160°C. However, it is difficult in these conditions to suppress the formation of discolouring components and bitter components. Accordingly, a preferred final heating temperature under the above reaction conditions is 140°C.

EXAMPLE 7

In this example a continuous vacuum evaporator was used. A palatinose solution in water was fed with a quantitative pump from a tank to vacuum heating tubes in which the solution was retained for about 1 minute, and then transferred to a vacuum evaporator in which the solution was retained for about 3 minutes. In the vacuum heating tubes, the aqueous palatinose solution flowed through the tubes and the outer wall of the tubes was heated at a predetermined temperature in the range of 125 of 150°C with high pressure steam. The concentration temperature in the vacuum evaporator was lower than the temperature of the vacuum heating tubes by about 10°C when the latter exceed 130°C or by about 6°C when the latter was 130°C or below. In every case, the amount of citric acid was 0.01% by weight and the

pressure was 50 mmHg. The results are as shown in Figure 6, where the ordinate is the content (% by weight) and the abscissa is the heating temperature of the vacuum heating tubes. The uppermost curve corresponds to palatinose in the heat concentration product; the middle the palatinose condensation product; and the lowest, to the total of the degradation product, the monosaccharide and the trisaccharide.

The amount of the condensation product formed was smaller in the continuous vacuum evaporator than that in the aforesaid batch mode where the heating time was 25 to 30 minutes, but the total amount of the degradation product, the monosaccharide and the trisaccharide which were formed was as very small as 4%, compared to that in the batch mode. Therefore, there is room to increase the amount of condensation product formed by raising the reaction temperature or by increasing the amount of organic acid added.

EXAMPLE 8

Preparation of palatinose hard jelly.

The sugar content is as high as 80% in a usual recipe of hard jelly. However, because palatinose crystallises easily, it was impossible to produce hard jelly containing it at such a concentration. Thus we have tried to produce palatinose hard jelly utilizing the palatinose condensation product.

The product mixture containing the palatinose condensation product used here was that obtained in Example 3 (solids content 99.1%). The used palatinose syrup was a syrup left after the crystallization of palatinose from a crude reaction mixture obtained by converting sucrose to palatinose with enzyme, the composition of which was as follows.

Composition of the palatinose syrup

| | |
|---|---|
| Solid | 71.1% |
| Glucose | 10.5% |
| Fructose | 9.2% |
| Sucrose | 2.5% |
| Palatinose | 13.8% |
| Trehalulose | 28.0% |
| Isomaltose | 3.7% |
| Other saccharides | 3.4% |

The blending ratio of raw materials for the palatinose hard jelly was as follows.

| Material | Blended amount (part) |
|---|---|
| Anhydrous citric acid | 14 |
| Pectin (super slow set Sag.150) | 22 |
| Powdery palatinose | 50 |
| Product mixture (from Ex. 3) | 834 |
| Palatinose syrup | 616 |
| Flavour | a little |
| Colourant | a little |
| Water | 322 |

Preparation.

The pectin and the powdery palatinose were mixed, transferred to a vacuum cooker and dissolved in water, which was then boiled over a fire and, subsequently, a sufficient vacuum was formed. The palatinose syrup and the product mixture were sucked through the sucking nozzle, and boiled down until the solid content became 80%. The vacuum was released and the heating was stopped, and then the 50% citric acid solution in water was added with vigorous stirring, followed by the addition of the flavour and the colourant. The mixture was poured into a mould.. After cooling, the jelly was

taken out of the mould, and crystalline palatinose was sprinkled on the jelly to produce a final product.

No crystallization was observed after this jelly was stored at room temperature for one month. Further, the taste was also excellent with rather low sweetness, and excellent texture and body. Accordingly, it is possible to produce excellent hard jelly using the palatinose condensation product of the invention instead of conventional malt syrup, and such jelly is less cariogenic and anti-cariogenic.

## EXAMPLE 9

Preparation of palatinose ice cream.

In general, sugar in ice cream often crystallizes during distribution to create an unfavourable texture. If palatinose is used, it is very difficult to prevent the crystallization because of its low solubility. In order to solve this problem, a palatinose ice cream has now been prepared using the palatinose condensation product. The product mixture and palatinose syrup used were the same as those used in Example 8. The blending ratio was as follows.

| Material | Amount (part) |
|---|---|
| Milk (fat 3.5%) | 40.0 |
| Cream (fat 40%) | 18.0 |
| Nonfat milk powder | 6.0 |
| Product mixture | 15.0 |
| Palatinose syrup | 7.0 |
| Emulsifier | 0.3 |
| Stabilizer | a little |
| Flavour | 15.4 |
| Water | |

Preparation.

The stabilizer and the emulsifier were dissolved

in water in a pasteurizer and heated to 70°C, followed by the proper addition of the remaining materials. This was then passed through a pressurized homogenizer and stored in a refrigerator. It was then placed in a freezer at -7°C, filled in cups and solidified in a solidifying vessel. The resulting product was subjected to a severe treatment test where the temperature was raised and lowered between -25°C and -3°C five times in 5 days. Precipitation of palatinose crystals was not observed. It was a good quality ice cream with plain and simple sweetness.

## EXAMPLE 10

Preparation of palatinose chewing gum.

In general, the amount of sugar in chewing gum is preferably from 0.1 to 3.9 times by weight to a gum base, and from 10 to 100 times to flavour for the continuation and buildup of flavour. However, if palatinose alone is used in chewing gum, viscoelasticity becomes insufficient resulting in damage to the texture. Therefore, a chewing gum has now been prepared in this Example by substituting the product mixture obtained in Example 1 for a part of the palatinose. The blending ratio was as follows.

| Material | Amount (part) |
|---|---|
| Sugar ester | 20 |
| Ester gum resin | 100 |
| Vinyl acetate | 120 |
| Wax | 80 |
| Peppermint flavour | 10 |
| Powdered palatinose (200 mesh) | 450 |
| Product mixture | 150 |
| Glyceride | 30 |

| | |
|---|---|
| Talc | 40 |

Preparation.

The above materials were mixed at 90°C and, after cooling, rolled and shaped into palatinose chewing gum of 3 g apiece. The chewing gum obtained had a continuing flavour and desirable texture.

When only palatinose was used as the saccharide, the upper limit of its amount for good viscoelasticity was 2 times by weight on the gumbase. However, it became possible to use a larger amount of palatinose by the aid of the palatinose condensation product as in the present Example.

## EXAMPLE 11

Bifidobacterium proliferation test with the palatinose condensation product.

From 29 volunteers, 8 persons who had a less quantity of Bifidobacterium in intestines were selected for the test. The eight volunteers were subject to restrictions on medicines and foods affecting intestinal flora during the experiments. The time period of the experiment was 40 days. In the first ten days, the composition useful for the proliferation of Bifidobacterium was not administered; in the 11th to 20th days, 4 candies (12 g) obtained in Example 4 containing 17.6% (= 18.1 x 0.97) of the palatinose condensation product were administered per day; in the 21st to 30th days, 8 candies (24 g) were administered per day; and in the remaining 10 days, no administration was conducted again. In every administration period, faeces of the volunteers were sampled every fi.e days and tested for the number of intestinal bacteria and their occupation ratios twice in every period. Table 5 shows the number

of bacteria in the unit of the number of each intestinal bacterium (common logarithm)/g faeces. Table 6 shows the occupation ratios of Bifidobacterium and other main bacteria to the total number of bacteria in the faeces (%). These results are average of all the subjects.

Table 5 Number of Bacteria

| Bacterium | Before administration | Administration of 12 g | Administration of 24 g | After suspension of administration |
|---|---|---|---|---|
| Enterobacteriaceae | 7.0± 0.8 | 7.0± 1.0 | 7.1± 1.1 | 7.2± 0.9 |
| Streptococcaceae | 7.4± 1.5 | 7.2± 1.5 | 7.6± 1.3 | 7.3± 1.1 |
| Staphylococcus | 3.0± 0.7 | 3.6± 1.0 | 3.3± 1.2 | 2.9± 0.5 |
| Yeast | 3.5± 0.9 | 3.3± 0.7 | 2.9± 0.5 | 3.0± 0.6 |
| Corynebacterium | 5.5± 1.1 | - | - | - |
| Bacillus | 2.6± 0.3 | 2.3 | 4.3± 1.7 | 4.2± 1.9 |
| Lactbacillus | 5.6± 1.7 | 6.0± 1.4 | 6.1± 2.0 | 6.3± 1.8 |
| Bifidobacterium | 9.5± 0.3 | 9.8± 0.4 | 10.0± 0.4 | 9.6± 0.5 |
| Eubacterium | 9.9± 0.3 | 10.0± 0.4 | 9.8± 0.5 | 9.8± 0.8 |
| Bacteroidaceae | 10.8± 0.2 | 10.8± 0.2 | 10.8± 0.2 | 10.8± 0.2 |
| Peptococcaceae | 9.8± 0.4 | 9.4± 0.5 | 9.6± 0.3 | 9.2± 0.8 |
| C. perfringens | 4.7± 0.5 | 4.7± 2.0 | 4.1± 1.9 | 5.3± 0.9 |
| Veillonella | 5.7± 1.7 | 4.8± 1.2 | 4.8± 1.6 | 4.5± 1.4 |
| Other Clostridia | 7.2± 1.2 | 6.4± 2.1 | 7.3± 1.1 | 7.4± 1.2 |
| Megasphaera | 8.2± 0.8 | 8.6± 0.3 | 8.9± 0.6 | 8.7± 0.6 |
| Total | 10.9± 0.2 | 10.9± 0.2 | 10.9± 0.1 | 10.8± 0.2 |

Table 6 Occupation Ratio of Main Bacteria

| Bacterium | Before administration | Administration of 12 g | Administration of 24 g | After suspension of administration |
|---|---|---|---|---|
| Bifidobacterium | 4.0 % | 7.9 % | 12.9 % | 6.3 % |
| Bacteroidaceae | 77.5 % | 75.8 % | 73.5 % | 80.6 % |
| Eubacterium | 10.0 % | 12.5 % | 7.9 % | 10.0 % |
| Peptococcaceae | 7.9 % | 3.2 % | 5.1 % | 2.5 % |

During the period of administration of the palatinose condensation product, the number of Bifidobacterium and its occupation ratio increased in seven persons out of the eight volunteers. The increase in the number of Bifidobacterium in comparison with that before the administration is statistically significant at the level of significance of 5% in the period of administration of 12 g, and at the level of significance of 1% in the period of administration of 24 g. The increase in the occupation ratio in comparison with that before the administration is statistically significant at the level of significance of 5% in the period of administration of 24 g. On the other hand, almost no change was observed in the other bacteria, and some bacteria show even a tendency of decrease. As the administration was suspended, the number of Bifidobacterium and its occupation ratio decreased. These results show that the palatinose condensation product specifically enhances the proliferation of Bifidobacterium.

EXAMPLE 12

Bifidobacterium proliferation test with palatinose condensation product.

Because Example 11 showed that the palatinose condensation product had the effect to enhance the proliferation of Bifidobacterium, further experiments were conducted on the seven volunteers of Example 11. Four candies (24 g) obtained in Example 1 containing 43.9% (= 44.3 x 0.99) of the palatinose condensation product were administered according to the same manner as in Example 11. In every administration period, faeces of the volunteers were sampled and tested for the number of intestinal bacteria and their occupation ratios in the similar-manner as in the above.

Table 7 Number of Bacteria

| Bacterium | Before administration | Administration of 24 g | After suspension of administration |
|---|---|---|---|
| Enterobacteriaceae | 7.3 ± 1.6 | 7.4 ± 1.4 | 7.5 ± 1.4 |
| Streptococcaceae | 7.1 ± 1.3 | 7.6 ± 1.0 | 7.3 ± 1.3 |
| Staphylococcus | 3.2 ± 0.6 | 3.6 ± 0.7 | 3.1 ± 0.7 |
| Yeast | 3.6 ± 0.6 | 3.3 ± 0.3 | 2.9 ± 0.4 |
| Corynebacterium | | | 7.3 ± 0.0 |
| Bacillus | 4.0 ± 1.9 | 5.1 ± 0.9 | 2.5 ± 0.1 |
| Lactbacillus | 6.5 ± 1.4 | 5.8 ± 1.9 | 5.6 ± 1.0 |
| Bifidobacterium | 9.3 ± 1.4 | 9.8 ± 0.9 | 9.1 ± 1.0 |
| Eubacterium | 10.1 ± 0.5 | 10.0 ± 0.4 | 10.2 ± 0.3 |
| Bacteroidaceae | 10.7 ± 0.2 | 10.6 ± 0.2 | 10.7 ± 0.2 |
| Peptococcaceae | 9.4 ± 0.4 | 9.2 ± 0.7 | 9.4 ± 0.3 |
| C. perfringens | 4.7 ± 0.9 | 4.7 ± 0.7 | 5.7 ± 1.2 |
| Veillonella | 5.5 ± 1.0 | 5.4 ± 1.6 | 5.1 ± 1.8 |
| Other Clostridia | 7.3 ± 2.4 | 8.3 ± 1.0 | 8.2 ± 1.0 |
| Megasphaera | 8.3 ± 0.0 | 8.5 ± 0.3 | 7.7 ± 0.7 |
| Total | 10.9 ± 0.2 | 10.9 ± 0.2 | 10.9 ± 0.2 |

Table 8 Occupation Ratio of Main Bacteria

| Bacterium | Before administration | Administration of 24 g | After suspension of administration |
|---|---|---|---|
| Bifidobacterium | 5.5 % | 23.3 % | 8.6 % |
| Eubacterium | 21.8 % | 17.9 % | 23.9 % |
| Bacteroidaceae | 65.5 % | 52.5 % | 62.3 % |
| Peptococaceae | 2.9 % | 4.0 % | 3.0 % |

In these experiments, the number of Bifidobacterium and the occupation ratio increased in six volunteers out of the seven volunteers during the period of administration of the palatinose condensation product. Particularly in five volunteers among them, the effect on the proliferation of Bifidobacterium was remarkable. The increases in number of Bifidobacterium and its occupation ratio during the administration in comparison with those before the administration were statistically significant at the level of significance of 5%. On the other hand, other major occupying bacteria, Bacteroidaceae and Eubacterium, showed the decreases in number and occupation ratio. The above results show that the palatinose condensation product is saccharide which is selectively assimilated by Bifidobacterium, but difficulty assimilated by other bacteria. That Bifidobacterium proliferates and, meanwhile, putrefactive bacteria decrease leads to the decrease of adsorption of ammonia noxious to a human body and the decrease of production of carcinogenic substances such as amines, phenols and indoles.

## EXAMPLE 13

### In-vitro test

In order to confirm the above results, a saccharide assimilation test by intestinal bacteria was conducted in vitro, where the fractionated palatinose condensation product was used instead of the product mixture.

The palatinose condensation product fractionated by chromatography was added in an amount of 5% to a 10% nonfat milk medium containing a pH indicator and homogeneously dissolved. A solution containing each of the intestinal bacteria was dropwise added to each medium,

covered with 2.5 ml of liquid paraffin and incubated at 37°C. The extents of the proliferation of Bifidobacterium and the other intestinal bacteria were determined by observing the change in colour of the indicator, from which the ability to assimilate the saccharide was judged. Besides, anaerobic incubation on the same media containing the palatinose condensation product was conducted in an anaerobic jar charged with carbon dioxide gas, and the change in pH of the media was observed.

Also in this test, the palatinose condensation product was well assimilated by Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium infantis and Bifidobacterium bifidum, but with other intestinal bacteria were clearly different in ability of the assimilation. That is, it has been found that only Bifidobactirium has high ability of exploiting the saccharide.

## EXAMPLE 14

Preparation of hard candy.

Each of the product mixtures obtained in Examples 2, 1 and 4 above was admixed with 1% of lyophilized black tea powder and 0.3% of an oil flavour on a cooling plate, which was moulded into hard candies of 3g apiece with a stamping machine.

These were subjected to an organoleptic test by fifteen panellists. The results are as follows.

| Palatinose condensation product from | Best physical properties | Best taste | Strongest sweetness |
|---|---|---|---|
| Example 2 | 4 | 0 | 0 |
| Example 1 | 11 | 10 | 2 |
| Example 4 | 0 | 5 | 13 |

## EXAMPLE 15

Preparation of hard jelly.

In the preparation of pectin hard jelly, the addition of the palatinose condensation product of 10to 70%, based on the whole saccharide, makes the viscosity increase and gives hard jelly of good quality. A hundred parts of hot water were added to 80 parts of a mixture of pectin (slow set Sag.150) and powdered sugar (1:9), and the pectin was completely dissolved while heating. Granulated sugar and each of the product mixtures of Examples 2, 1 and 4 were mixed so that the weight ratio of the granulated sugar to the palatinose condensation product was 1:3, 1:1 or 3:1. Four hundred parts of each of the mixtures were added to the above solution to dissolve, and the heating was stopped. Then, a small quantity of a flavour and a colourant, and 2 parts of citric acid were added, and the mixture was then poured into a mould.

The product was subjected to an organoleptic test by fifteen panellists. The results are as follows.

| Palatinose condensation product from | 1 : 3 | | 1 : 1 | | 3 : 1 | |
|---|---|---|---|---|---|---|
| | Taste | Appearance | Taste | Appearance | Taste | Appearance |
| Example 2 | good | slight discoloring | slightly bitter | discoloring | bitter | discoloring |
| Example 1 | good | no discoloring | good | no discoloring | good | slight discoloring |
| Example 4 | good | no discoloring | good | slight discoloring | good | discoloring |

## EXAMPLE 16

Preparation of refreshing drink.

In the preparation of a refreshing drink suitable to be mixed with Shochu (a kind of distilled spirits), a less sweet, and rich refreshing drink was obtained by adding the palatinose condensation product of 3 to 10% as saccharide. Each of the product mixtures of Examples 2, 1 and 4 was dissolved in an amount of 10%, calculated as the palatinose condensation product, in soda water, to which a small quantity of a flavour and 0.1% of citric acid were added. For comparison, 2.5% of sucrose were added instead of the palatinose condensation product. The amount of sucrose, 2.5%, was such as to give mild sweetness.

Eighty (80)parts of the above blend and 20 parts of ice were added to 20 parts of Shochu (distilled spirits, 25°), which was then subjected to an organoleptic test by fifteen panellists. The results are as follows.

| Palatinose condensation product from | Ranking of preference | Major remarks |
|---|---|---|
| Example 2 | 1 | mild as a whole, easy to drink, and full of body |
| Example 1 | 2 | a little too sweet, but mild and easy to drink |
| Example 4 | 3 | a little too sweet, but mild and full of body |
| Sucrose 2.5% | 4 | no balance among tastes |

## CLAIMS

1. A palatinose condensation product composed of 2 to 6 palatinose units.

2. A palatinose condensation product composed of 2 to 4 palatinose units.

3. A palatinose condensation product hydrolysable substantially to palatinose.

4. A mixture of palatinose and a palatinose condensation product which has a reduced tendency to crystallization of palatinose as compared to a composition containing the same amount of palatinose but no palatinose condensation product.

5. A mixture of palatinose and palatinose reaction product as claimed in any one of Claims 1 to 3.

6. A palatinose reaction product or a mixture was claimed in Claim 5 substantially as specifically described herein with reference to the accompanying examples.

7. A process for the preparation of a palatinose condensation product in which an aqueous palatinose solution having a pH of about 3.2 to 5.8 is heat-concentrated up to a liquid temperature of about 105 to 170°C.

8. A process for the preparation of a palatinose condensation product in which an aqueous palatinose solution is heat-concentrated up to a liquid temperature of about 140 to 165°C at atmospheric pressure.

9. A process for the preparation of a palatinose condensation product in which an aqueous palatinose solution is heat-concentrated up to a liquid temperature of about 105 to 160°C at pressures of about 110 mmHg or below.

10. A process as claimed in any one of Claims 7 to 9 in which the pH of the aqueous palatinose solution is adjusted with one ormore less volatile organic acids.

11. A process as claimed in Claim 10 in which the acid is citric acid, malic acid, succinicacid or tartaric acid or a mixture thereof.

12. A process as claimed in any one of Claims 7 to 11 substantially as specifically described herein with reference to the accompanying examples.

13. A palatinose condensation product whenever prepared by a process as claimed in any one of Claims 7 to 12.

14. A reaction mixture obtained from a process as claimed in any one of Claims 7 to 12.

15. A food product containing a material as claimed in any one of Claims 1 to 6 or 13 or 14.

16. A food product as claimed in Claim 15 also containing palatinose.

17. A food product as claimed in Claim 15 substantially as specifically described herein with reference to the accompanying examples.

18. A medicament containing a material as claimed in any one of Claims 1 to 6 or 13 or 14.

19. The use of a palatinose condensation product in the preparation of a product for oral administration to encourage Bifidobacterium.

20. A process for encouraging the proliferation of Bifidobacterium which comprises administering a material as claimed in any one of Claims 1 to 6 or 13 or 14 to an environment containing Bifidobacterium.

21. Bifidobacterium whenever produced by a process as claimed in Claim 20.

22. A composition useful in the proliferation of Bifidobacterium, containing a palatinose condensation product in an effective amount.

23. A composition as claimed in Claim 22 in which the palatinose condensation product is a material as claimed in any one of Claims 1 to 6 or Claim 13 or 14.

24. A composition as claimed in Claim 22 or Claim 23, containing about 0.5 to 80% by weight, calculated on the composition, of the palatinose condensation product.

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